ANNUAL REPRODUCTIVE CYCLE OF THE MALE POCKET GOPHER (*Geomys pinetis*)

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Chairman: David W. Johnston
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An analysis was made of the reproductive cycle of the southeastern pocket gopher, *Geomys pinetis*, to determine both the pattern of reproduction in this species and the magnitude of environmental influences on reproduction in a fossorial habitat near Gainesville, Florida. The primary focus of the investigation was the male reproductive cycle, but gross measurements of the reproductive condition of the females were also used.

The right testis, its epididymis, and the proximal accessory glands (seminal vesicles and prostate glands) of 76 male pocket gophers collected monthly between March, 1967, and July, 1968, were sectioned and examined histologically to assess the degree of reproductive activity of each organ. A one-way analysis of variance was performed on each of the measurements made on the various organs to determine if a monthly cycle of activity was present. A principal components analysis was also performed to find out what interrelationships might exist between the parameters. Finally, a canonical analysis related those parameters which showed cyclical differences

during the trapping period with corresponding soil temperature and soil moisture values to compare the effects of the environmental parameters on the reproductive cycle.

During a subsequent twelve-month trapping period, quarterly population samples were made in the same study area. Only gross measurements of the reproductive condition were made on the 60 individuals in this part of the investigation.

The reproductive cycle in the male pocket gopher was found to be closely correlated with soil temperature and to a lesser degree with soil moisture. Maximum reproductive activity occurs when environmental conditions create a friable soil, suggesting an increased likelihood of intraspecific contacts. Very warm external temperatures and low soil moisture inhibit reproductive activity indirectly, presumably by restricting activity within the burrow.

Leydig cells show a net decrease in size and number with age, after an initial increase at puberty. Increased reproductive activity is correlated with an increase in Leydig cell diameter. Spermatogenesis was non-cyclic, but did not cease in the entire population during the study period. The activity of the accessory glands and the epididymis served as a more reliable index of an individual's reproductive condition.

INTRODUCTION

Fossorial rodents are unique among terrestrial mammals in being isolated from most of the environmental factors that control daily and annual biological cycles in many other organisms. Typical of these cycles is reproduction, which may be controlled or influenced in many mammals by the available food supply, temperature, photoperiod, or rainfall. In studying reproduction of a fossorial mammal, these external factors must be translated to their effects on the environment of the burrow. In this investigation, emphasis is given to an analysis of environmental effects on the reproductive cycle of a fossorial rodent, the southeastern pocket gopher (Geomys pinetis Rafinesque).

This animal spends almost its entire life underground and is well adapted to a fossorial existence, as indicated by its relatively broad, flattened skull, small eyes and pinnae, stout forelimbs, and elongated foreclaws. It feeds chiefly on roots obtained from its burrow, as well as on occasional leaves pulled into the shallower parts of the burrow. It is seldom seen above ground, and is exposed to daylight only during short periods when making fresh mounds. Presumably, pocket gophers are influenced more by the environment of the burrow than by light. Particularly important influences would include temperature changes, gas composition, and soil characteristics.

Pocket Gopher Environment and Physiological Adaptations

Kennerly (1964) found that the temperature within the burrow of Geomys bursarius in central Texas is more constant than environmental temperatures, and that the range of burrow temperatures becomes narrower at increased depths. McNab (1966) noted that temperature changes within burrows of G. pinetis in Florida are also less extreme than outside temperatures. Because of the temperature stability in the burrow, Wilks (1963) reasoned that there might be less selection pressure towards strict homothermy. Poor thermoregulation has indeed been found to be characteristic of fossorial rodents (Gunther, 1956; Wilks, 1963; McNab, 1966). McNab (1966) claimed that conductance is facilitated by a scanty fur coat, an increase in peripheral circulation, and a general decrease in body size and mean weight in areas of higher soil temperature. The greater stress of summer temperatures, accentuated by the burrow's saturated atmosphere (McNab, 1966), might still pose a problem; the pocket gopher could, however, avoid heat loading by retreating into the deeper, cooler part of its burrow system. From 10 to 20% of the burrow systems of Thomomys bottae and G. bursarius lie well below the superficial runways, which are within 12 inches of the surface (Miller, 1964).

The oxygen and carbon dioxide concentrations in the burrow system fluctuate more widely than does temperature. Increased soil moisture causes the carbon dioxide content, which is initially several times greater than the atmospheric concentration, to rise even more, probably due to decreased diffusion through the soil pores (Kennerly, 1964). As a result, according to measurements made in Florida by McNab (1966),

the carbon dioxide concentration in the burrow reaches as much as 20 to 35 times the atmospheric concentration. The oxygen concentration remains within 80 to 95% of the atmospheric concentration.

Effects of Environment on Activity Cycle

Little is known about the influence of soil moisture and soil temperature on pocket gopher activity. No published data on activity cycles within the burrow are available, and Vaughan and Hansen (1961) remarked that the usual indicator of activity -- the appearance of fresh mounds -- is really only indicative of surface or near-surface activity. Trapping experience, however, indicated to these authors and to Wilks (1963) that soil temperature is an important factor controlling the pocket gopher's daily activity cycle.

Miller (1948) found that the amount of burrowing (determined by the appearance of fresh mounds) is primarily controlled by soil moisture, the greatest activity occurring when the soil was most friable. Kennerly (1964) came to the same conclusion, but the correlation seemed weaker to him.

Reproductive Cycles in Pocket Gophers

Reproductive activity in most species of pocket gophers is limited by their usually solitary behavior, although instances of multiple captures within one burrow system have been reported for many species of Geomys and Thomomys (e.g., English, 1932; Vaughan, 1962; Wilks, 1963; Miller, 1964). Both Vaughan (1962) and Wilks (1963) observed that these multiple captures were most frequent during the breeding season and usually involved animals of opposite sexes.

Wing (1960) listed only one multiple capture in G. pinetis, involving an adult male and a post-partum female. She also cited 14 instances during one year of collecting in which a pocket gopher was caught in one trap when the second trap had been sprung. This, however, could have been accomplished by one animal's blocking one trap before being caught in the other. In general, it appears that pocket gophers remain solitary within their burrows most of the year.

Ecological aspects of reproductive cycles have been studied in several species of pocket gophers. In east Texas, English (1932) showed that only one litter per year was characteristic of G. breviceps, whereas Wilks (1963) reported at least two litters per year for G. bursarius in south Texas. Peaks of reproductive activity in G. bursarius were found in December - January, and April - May, with some activity in July - September. Wilks suggested that temperature, humidity, and rainfall were interacting to inhibit summer breeding. G. bursarius in Colorado produced litters only in the spring (Vaughan, 1962). In this case, the breeding season seemed to be timed so that the young appeared in late May or June at the peak of vegetative growth and when the soil was most friable. The adults began to come into reproductive condition in late winter while the ground was still frozen to a depth of six inches.

According to Miller (1946), T. bottae in California has only one breeding season in the northern, more mountainous part of its range, whereas the breeding season in the southern part seemed to extend through part of the winter rainy season to allow two or three litters per year. Dixon (1929) felt that this cycle was attuned primarily to the appearance of the pocket gopher's most important food plants at

the beginning of the season. He found that in the central San Joaquin Valley, where alfalfa and other green forage crops were available throughout the summer, the breeding season was longer. Gunther (1956) also described the breeding season of this species as restricted to the cool, wet weather of winter and spring in nonirrigated portions of the range, but being more protracted in irrigated areas. Bond (1946) suggested that whereas availability of food might affect the sexual development of a pocket gopher, contact between the sexes at the optimum time for reproduction depended more on the friability of the soil.

Wing (1960) described the breeding cycle of G. pinetis in Florida, but made no correlations with environmental factors. Pregnant females were encountered in her collections throughout the year, but they appeared more frequently in early spring and late summer. Probably some females had produced at least two litters per year, since double sets of placental scars occurred in seven females collected between April and July. Her work included a thorough examination of the females as well as gross measurements of the male reproductive tracts and epididymal sperm smears. The males in her samples showed greatest reproductive activity from January - August, whereas females were most active in March and July - August.

The Reproductive Cycle of Geomys pinetis

Unlike other species of pocket gophers that inhabit temperate regions, G. pinetis does not appear to have a well-defined breeding cycle that can be easily attributed to environmental effects. Wing has suggested that breeding continues throughout the year but not at the same intensity. There are at least four different ways in which

the male reproductive cycle could be operating to generate this pattern: 1.) The males may remain in an active reproductive state all year, but the females come into breeding condition only at presumably propitious times. 2.) The males do not remain in an active state of spermatogenesis all year, but instead store sperm for a certain period of time after spermatogenesis has ceased, meanwhile retaining a propensity toward mating. 3.) The males may show fluctuations due to environmental conditions, although never completely losing the ability to reproduce. 4.) Some males may maintain at least a threshold ability to reproduce whereas others regress significantly; a well-defined cycle is therefore not maintained in the population.

The purpose of this investigation was to determine which reproductive pattern exists for G. pinetis near Gainesville, Florida, and to examine in greater depth the influence of environmental factors.

MATERIALS AND METHODS

Study Area and Trapping Methods

At least five male pocket gophers were trapped every month from March, 1967, through July, 1968, within a five-mile radius of Gainesville, Florida. After July, 1967, all trapping was done at Stengel Field, three miles WSW of Gainesville. This field, containing a fairly large population of pocket gophers, was characterized by Pensacola bahia grass sod over Arredonda fine sand and was mowed fairly regularly throughout the trapping period. Part of the field was used as an unpaved air strip. The animals were captured in Victor gopher traps which were checked every two to three hours to avoid excessive decay of the tissues in the dead animals.

In addition, samples of 15 pocket gophers, including both sexes, were trapped in November, 1968, and in February, May, and August, 1969, to obtain quarterly population samples in Stengel Field.

A total of 106 males and 91 females was obtained for analysis in the present investigation.

Environmental Data

Climatological data were obtained from the Agronomy Farm Weather Station, an area one mile east of Stengel Field and quite similar to it in soil characteristics and cover. The temperatures used in the statistical analyses were soil temperatures at a depth of four inches

and were averages between the daily maxima and minima. Estimates of soil moisture were obtained by combining weekly estimates of evapotranspiration with total weekly rainfall. Field capacity, defined as the ability of a soil to hold water against the pull of gravity, is 13.52% by volume for the top two feet of Lakeland fine sand (Hammond et al., 1967). Hammond (pers. comm.) estimated that this value is probably very similar to the figures for the Arredonda fine sand in the trapping area and at the Agronomy Farm. With rainfall as input and evapotranspiration as output, soil moisture was calculated for each week beginning at a time when field capacity had been obtained. Evapotranspiration values were obtained from measurements that had been taken daily on two lysimeters at the Agronomy Farm. Daily output (leachate) was subtracted from input (2000 mm of irrigation water daily plus any rainfall) to give a rough indication of soil moisture (Tosi, 1968). This method should be reliable when used on a weekly basis, and should certainly provide a relative indication of periodic soil moisture cycles.

Characteristics of Reproductive Organs

Certain standard measurements were taken of all specimens: lengths of the body, tail, hind foot, and ear, and body weight. Testis length and width, color, and vascularity were recorded for males. Mature males were distinguished from immature males by a testis volume larger than 1000 mm^3. This value was selected after comparing the logarithm of the testis volume with the logarithm of the body weight for all male pocket gophers (Fig. 1). Spencer (1968) found that the relationship between the growth rates of the testis and the whole body was

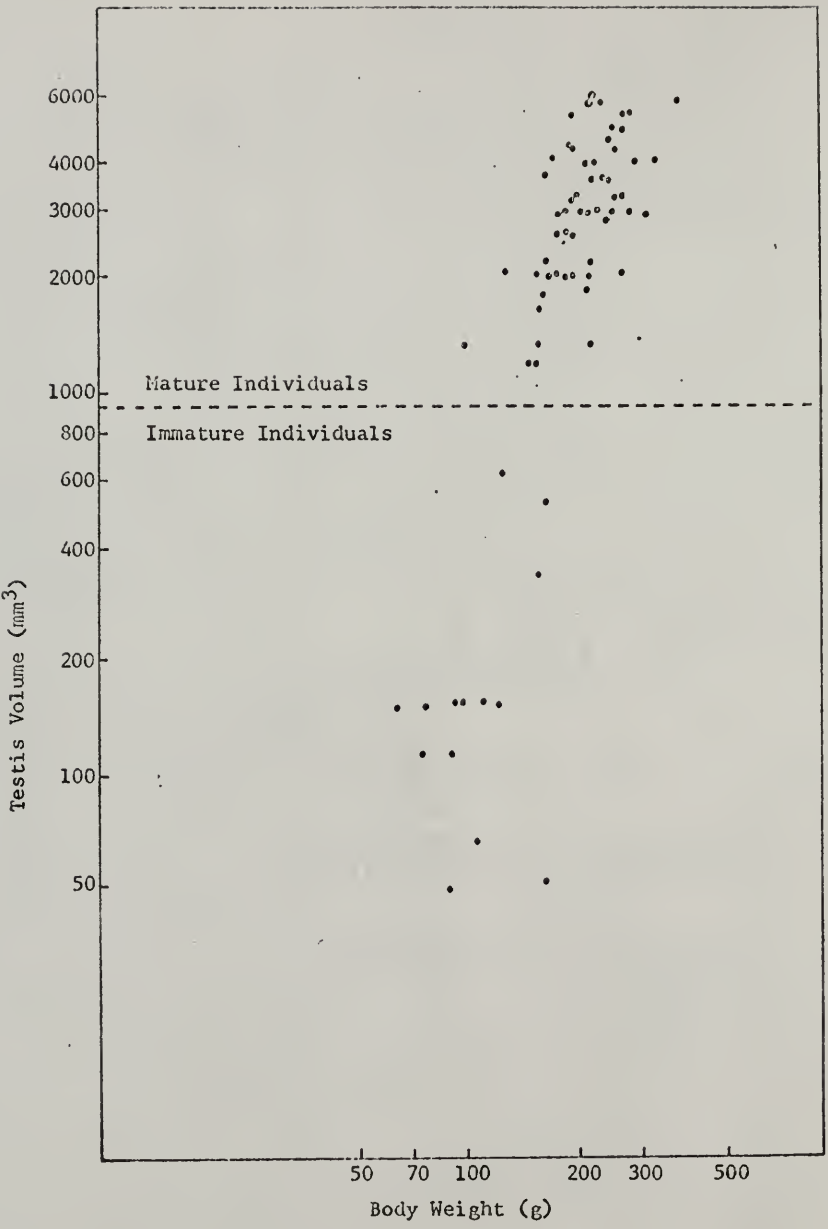

Fig. 1. Criterion for separating mature from immature male pocket gophers

altered at puberty, and he noted that this was reflected in a change in the log - log slope of organ weight vs. body weight. When the testis volume and body weight for all pocket gophers were compared on a logarithmic scale, two distinct clusters appeared, separated by a difference of nearly 900 mm^3 in testis volume measurements. Three individuals intermediate between the clusters were classified as immature males, because measurements of the epididymal tubules and the accessory glands were infantile.

Reproductive conditions of females were defined by conditions of the nipples (furred or exposed), the uterus (degree of vascularity and swelling) and the pubic bone (the symphysis is resorbed at puberty (Hisaw, 1923)).

Histological Methods

Sections were made of various reproductive organs. The right testis, cauda epididymis, and the entire proximal set of accessory glands (prostates and seminal vesicles) from each male were preserved in Bouin's fixative. After being embedded in paraffin, these organs were sectioned at 10 μ and stained with Heidenhain's iron-hematoxylin. Sections were made from regularly spaced intervals throughout the length of the testis and the epididymis. Serial sections of the accessory glands were prepared to facilitate tracing the tubules. However, with enlarged sets of glands, the sections were again chosen at intervals to prevent an accumulation of large numbers of slides.

Examination of the Testis

Several criteria were used to determine the reproductive condition of the testis. The initial gross measurements included the lengths of the two axes of the testis, as though it were a perfect ellipsoid. The volume was then determined by the equation: $V = 4/3\ \pi\ ab^2$, "a" being the long axis and "b" the short axis.

An average value for testis tubule diameter was calculated for each individual from ten randomly selected round tubules.

The spermatogenic values of twenty tubules, randomly selected among the round tubules in several sections, were also determined according to the following scale adapted from Roosen-Runge and Giesel (1950), with some modifications proposed by Johnson and Buss (1967):

Level 1. Little cell differentiation, no lumen, sparse germ cell population

2. Immature tubule with few primary spermatocytes

3. Mostly primary spermatocytes, some secondary spermatocytes, few spermatids

4. Much spermatid formation

5. Spermatids undergoing elongation but not yet in bundles

6. Spermatids in bundles moving toward tubule periphery

7. Spermatozoa moving from periphery to lumen; "wheel-spoke" stage

8. Spermatozoa lining lumen

An "active" testis would have a certain proportion of tubules in Levels 3 - 8, since the Level 8 tubules revert to Level 3 after losing

their spermatozoa. Inactive testes, i.e., those lacking tubules that are actively producing sperm, may have Level 1 and Level 2 tubules as well as Level 3 and occasionally Level 4 tubules.

The specific stage of spermatogenesis shown by each animal was determined according to the following characteristics:

Stage 0. No spermatocytes, totally inactive
1. Primary spermatocytes present
2. Secondary spermatocytes present, some spermatids
3. Many spermatids in Level 4
4. Most advanced spermatids in Level 5
5. Most advanced spermatids in Level 6
6. Spermatozoa present in Level 7 only
7. 5% of tubules contain spermatozoa in Level 8
8. 10% of tubules contain spermatozoa in Level 8
9. 15% of tubules at Level 8
10. 20% of tubules at Level 8
11. 25%
12. 30%
13. 35%
14. 40%
15. 45%

Since the Leydig cells in the interstitial tissue are believed to secrete testosterone, the male hormone that controls the activity of the secondary sex characteristics (Bloom and Fawcett, 1962), those Leydig cells with visible nuclear and cell membranes were measured. Both the cell diameter and the nuclear diameter of round cells were recorded at 1000X.

The total volume of Leydig cells in the testis was obtained by the following method, after Groome (1940). Using an ocular grid measuring 1.92×10^{-4} mm^2 at 1000X and focusing through the 10 μ depth of the section, the total number of Leydig cells in several randomly selected fields was counted. Since the volume of each field was known (1.92×10^{-6} mm^3), the average number of cells per field was multiplied by the number of field-volumes contained in that particular testis to get the total number of Leydig cells in the testis. The average Leydig cell diameter in each animal was used to calculate the total volume of the Leydig cell tissue in the testis; this value was divided by the testis volume for a measure of the percentage of the total volume.

Examination of the Epididymis

The epididymis consists of three regions: the caput epididymis, which contains both the epididymal duct and efferent ducts from the rete testis; the corpus epididymis; and the cauda epididymis. The tubule diameter, cell height, and nuclear diameter were each averaged over ten tubules in the cauda epididymis.

Examination of the Accessory Glands

Each ductus deferens receives secretions from the seminal vesicles and the prostate glands before joining the urethra.

The seminal vesicle in the young animal and in the inactive adult is strongly lobulated, but the cavities and recesses essentially disappear during active secretion, when lumen expands. The cells in the recesses appear to be the most active secretory units (Moore et al.,

1930); ten such cells were measured among the sections from each individual to give an average height, and the relative amount of secretion present in the lumen of the vesicle (0, 1, or 2) was noted. If no secretion was found, the rating was 0. A trace amount was given a rating of 1, and the presence of enough secretion to cause distension of the vesicle was rated as 2.

The prostate gland is divided into three sections: dorsal and lateral units, which are considered as one unit here, and the ventral unit, which is interior and anterior to the others and seems to be much less extensively developed (Macklin and Macklin, 1963). Ten cells from acini of each of the two sections were measured and the relative secretory activity was also recorded as in the seminal vesicle.

Statistical Analysis of the Data

Correlation coefficients were calculated for all possible pairs of measurements (including 16 reproductive parameters and four environmental parameters) by eliminating from the calculation of a given coefficient those individuals which lacked either or both of the measurements. A program from the University of Florida Statistical Program Library, "Intercorrelation with Missing Data" (UFSPL020), computed these coefficients. This correlation coefficient matrix served as input for the Biomedical Computer Program, "Factor Analysis" (BMD03M). Because the correlation coefficients were used as the input rather than the raw data, this program provided only a principal components analysis. The technique reduces the correlation coefficient matrix to a smaller number of uncorrelated

variables, or factors, which group the most similar parameters. Each of the original variables is weighted according to the strength of its relationship with all the other variables in each factor. The most important factor is the first, since it contains the greatest proportion of the total variance.

Since the trapping was divided into 15 periods, a one-way analysis of variance was performed on each of the parameters (BMD01V, "Analysis of Variance for One-Way Design"). The means of those parameters showing significant F ratios at the .05 level of significance were then tested for significance, again at the .05 level, by Duncan's New Multiple Range Test adjusted for unequal sample sizes (Steel and Torrie, 1960).

Parameters showing significant differences (i.e., cyclical changes over the 16-month trapping period) were grouped together and compared with a set of environmental parameters in a second multivariate technique, canonical analysis (BMD06M, "Canonical Analysis"). Only the 31 individuals with complete sets of data for the significant parameters were used. For each individual, the average soil moisture and soil temperature values for the two-week and four-week periods previous to the date of capture were calculated. The canonical analysis treated the reproductive parameters as one set of data, the environmental parameters as another. Two linear functions of the general form: $Y = a_1x_1 + \ldots + a_nx_n$ were computed for each set, such that Y_1 and Y_2 were maximally correlated. The "a" coefficients give the relative importance of each parameter, x, in calculating the coefficient Y.

Four canonical analyses were run, each program including the same reproductive data but with a different combination of soil tem-

perature and soil moisture values. Thus data on soil temperature for the two weeks prior to capture were paired with the soil moisture for two weeks prior in one program and four weeks prior in the second program. The data on soil temperature for four weeks prior were similarly paired with each of the soil moisture sets in the last two programs. Two independent canonical correlations were calculated in each program, each correlation maximizing a different environmental parameter. Each successive correlation was less significant than the preceding one.

Glandular activity between the different kinds of glands and in individuals of varying degrees of reproductive activity were compared using the chi-square criterion at the .05 level of significance.

RESULTS

Noncyclic Changes in Reproductive Organs

While the intent of this investigation was to study the cyclic changes in reproductive activity, it was obvious that non-cyclic, or age-dependent, changes might also be occurring. Table 1 lists the differences between the means of measurements in mature and immature animals, but gives no indication of what further variations might occur after puberty has been reached. Changes in one organ may also be correlated with changes in another organ or group of organs; this may be an important factor that is missed by studying the isolated parameters. The principal component analysis provided a means of looking at the set of parameters as a whole, rather than individually. It selected from the entire set of measurements from mature animals a small number that accounted for most of the intercorrelations of the larger set.

Three groupings, or factors, emerged from this analysis (see Table 2). Factor 1 was significant in these considerations because of the high loading given to body length. This parameter would obviously not have fluctuated from season to season, and, therefore, was a measure of age. The correspondingly high loadings on those parameters whose values are starred in Table 2 under Factor 1 indicate that they change with age.

Table 1. Means of measurements from male pocket gophers

Parameter	Immature	N	Mature	N
Body Length (mm)	141.0	14	178.9	61
Body Weight (g)	106.2	13	214.5	62
Testis Volume (mm^3)	219.4	14	3282.8	59
Testis Tubule Diameter (u)	66.8	11	160.4	61
Stage of Spermatogenesis	1.1	11	9.3	61
Leydig Cell Diameter (u)	5.6	11	8.2	59
Nucleus-Cell Ratio of Leydig Cell (%)	84.4	11	67.4	59
Leydig Cell Tissue Volume (mm^3)	21.0	11	370.2	55
Leydig Cell Tissue, % Testis Volume	9.9	11	11.3	55
Epididymal Tubule Diameter (u)	48.5	9	226.3	52
Epididymal Cell Height (u)	11.3	9	22.5	52
Epididymal Cell-Tubule Ratio (%)	23.6	9	12.5	52
Seminal Vesicle Diameter (mm)	0.4	7	1.9	52
Seminal Vesicle Cell Height (u)	11.5	7	17.5	54
Dorsolateral Prostate Cell Height (u)	9.3	12	17.4	51
Ventral Prostate Cell Height (u)	10.1	7	14.9	36

Table 2. Independent factors in measurements from male pocket gophers

Parameter	Factor 1	2	3
Body Length	.8553*	.0717	-.0244
Body Weight	.1373	-.0403	.1853
Testis Volume	.5449*	.1625	.2069
Testis Tubule Diameter	.8860	-.0316	.2910
Stage of Spermatogenesis	.9322*	-.0521	.1934
Leydig Cell Diameter	-.6497*	.1868	.7099*
Nucleus-Cell Ratio of Leydig Cell	-.7208*	-.4241*	-.0321
Leydig Cell Tissue Volume	.3342	.8337*	.1660
Leydig Cell Tissue, % of Testis Volume	-.1238	.9373*	.0600
Epididymal Tubule Diameter	.3919	-.0199	.4130*
Epididymal Cell Height	-.1932	-.1202	.1307
Epididymal Cell-Tubule Ratio	.5357*	.1172	.5303*
Seminal Vesicle Diameter	.0348	-.2060	.4868*
Seminal Vesicle Cell Height	.1755	.0785	.8616*
Dorsolateral Prostate Cell Height	.1841	.0575	.8621*
Ventral Prostate Cell Height	.1935	.1201	.7572*

*indicates most important loadings

Most of the measurements increased in magnitude with age. The Leydig cell diameter decreased, however, as did the proportion of the nucleus to the cytoplasm. The loading for Leydig cell tissue volume may not have been significant, but its magnitude suggested that this parameter also increased with age. The percentage of volume taken up by Leydig cells did not increase, however.

Factor 2, which has no relationship to age or to Factor 1, showed that Leydig cell tissue volume and the percentage of Leydig cell tissue in the testis increased with an increase in the proportion of cytoplasm to nucleus in the Leydig cell. Since Factor 1 did not associate increase in percentage of Leydig cell tissue in the testis with age, but did show that the nucleus-cell ratio increased with age, Factor 2 suggested that there was actually a cyclic increase and decrease of both tissue and cell volume.

In Factor 3, the Leydig cell diameter increased with glandular activity in each of the accessory glands that was studied. Leydig cell diameter was also related by this factor to the epididymal cell height, which increased disproportionately to the epididymal tubule diameter with age. This was also reflected in the high loading in Factor 1, indicating that the epididymis cell increased in height in relation to the tubule size.

The increase in testis volume was attributable primarily to the increase in size of the testis tubules, as indicated by the decrease in percentage of Leydig cell tissue. The high loading on spermatogenesis in Factor 1 showed that active sperm production was not cyclic in the full-grown adult, but became more or less constant.

Interrelationships among activity levels in the accessory glands were considered important even though they did not appear in the factor analysis. The measurements of activity were all significantly correlated with one another. However, comparison by means of a chi-square test of the relative amounts of secretion (absent = 0, slight = 1, substantial = 2) in the acini or vesicles examined showed that within a single animal there was no significant difference at the .05 level between the proportions of acini (and of secretory units in the seminal vesicles) at each stage of secretory activity in the various glands (Table 3). The accessory glands therefore appeared to secrete as a unit, rather than varying individually.

A comparison of glandular activity was also made between those adults with active sperm in their testes (Stages 7 - 15) and those without active spermatozoa. The proportion of dorsolateral prostate acini in each of three stages of secretion was not significantly different between the two groups. Some secretion in the seminal vesicle also took place in both groups, but there was significantly greater secretion in those animals with active sperm. These results are shown in Table 4.

Cyclic Changes in Reproductive Organs

The 16-month total trapping period was broken up into 15 separate periods lasting from one day to as much as 31 days, the average length being 10 days. At least two weeks usually separated each trapping period from the adjacent ones. The periods are defined in Table 5.

Table 3. Numbers of pocket gophers with acini at a given stage of secretory activity in the prostate glands

Stage of Secretory Activity in Seminal Vesicle	Stage of Secretory Activity: Dorsolateral Prostate			Stage of Secretory Activity: Ventral Prostate		
	0	1	2	0	1	2
0	0	2	0	0	0	0
1	1	5	4	1	4	4
2	0	14	28	2	17	5
	$X^2 = 9.435$			$X^2 = 2.108$		
	$X^2_{.05,8} = 15.15$			$X^2_{.05,8} = 15.15$		

Table 4. Relationships between the production of sperm and accessory gland activity. The pocket gophers are grouped according to their average stages of secretory activity and their stages of spermatogenesis.

	Stage of Secretory Activity					
	Seminal Vesicle			Dorsolateral Prostate		
Stages of Sperm Production	0	1	2	0	1	2
0 - 6	3	7	4	0	9	5
7 - 15	0	3	36	1	9	27
	$X^2 = 23.67$*			$X^2 = 9.868$		
	$X^2_{.05,5} = 11.1$			$X^2_{.05,5} = 11.1$		

*indicates significance at the .05 level

Table 5. Number of males caught in each trapping period

Trapping Period	Duration	Total Number of Males Caught	Total Number of Mature Males Caught
1	3/19/67 - 4/16/67	6	6
2	5/11/67 - 5/31/67	5	4
3	6/8/67 - 7/8/67	6	4
4	8/1/67 - 9/1/67	6	5
5	9/14/67 - 9/28/67	5	2
6	10/12/67 - 11/7/67	6	5
7	11/25/67 - 11/25/67	3	3
8	12/7/67 - 12/29/67	4	4
9	1/11/68 - 1/25/68	5	4
10	2/13/68 - 2/17/68	5	5
11	3/7/68 - 3/13/68	6	5
12	4/14/68 - 4/25/68	4	4
13	5/16/68 - 5/21/68	5	3
14	6/8/68 - 6/10/68	5	5
15	7/8/68 - 7/9/68	5	3

Table 6 lists the results of the analysis of variance test run on each of the reproduction parameters. The F ratios are listed in descending order of significance, and are starred according to whether or not differences between the means of each period were significant.

Changes in the Testis

Changes in testis volume throughout the year are shown in Fig. 2. Multiple range tests showed that the peaks in Periods 3 and 11 were significant, but the peak in Period 7 was not significantly different from the lows in Periods 5, 6, and 9, perhaps because it was a mean of only two values. It will be considered significant here, however, primarily because of the significance of the corresponding peak, which included a third individual, in the cycle of testis tubule diameters (Fig. 3). Period 8 also contributed to the first significant peak in Fig. 3. The second significant peak extended from Period 11 through Period 13.

Changes in Leydig cell diameter are outlined in Fig. 4. There are significant peaks at Periods 3 and 11. The changes in the ratio of nuclear diameter to cell diameter in Leydig cells were also tested and found to be significant. The resulting pattern was essentially a mirror image of the pattern in Fig. 4, although the decrease in the ratio (reflecting an increase in cell diameter) was significantly low from Periods 11 - 13.

Significant peaks in Leydig cell tissue volume (Fig. 5) appeared in Periods 1, 3, and 11. The peak in Period 7, again a mean of two values, was not statistically significant, but will be considered significant for the same reasons given above.

Table 6. F ratios from univariate analyses of variance showing significance of differences between means of reproductive parameters in 15 trapping periods

Parameter	F
Epididymal Tubule Diameter	4.45**
Testis Tubule Diameter	4.36**
Leydig Cell Diameter	4.04**
Seminal Vesicle Cell Height	3.63**
Epididymal Tubule-Cell Ratio	3.57**
Seminal Vesicle Diameter	2.53*
Dorsolateral Prostate Cell Height	2.52*
Leydig Cell Tissue Volume	2.30*
Nucleus-Cell Ratio of Leydig Cell	2.16*
Testis Volume	2.03*
Ventral Prostate Cell Height	2.03
Body Weight	1.63
Stage of Spermatogenesis	1.38
Epididymal Cell Height	1.31
Leydig Cell Tissue, % of Testis Volume	1.00

*indicates significance at the .05 level of significance
**indicates significance at the .01 level of significance

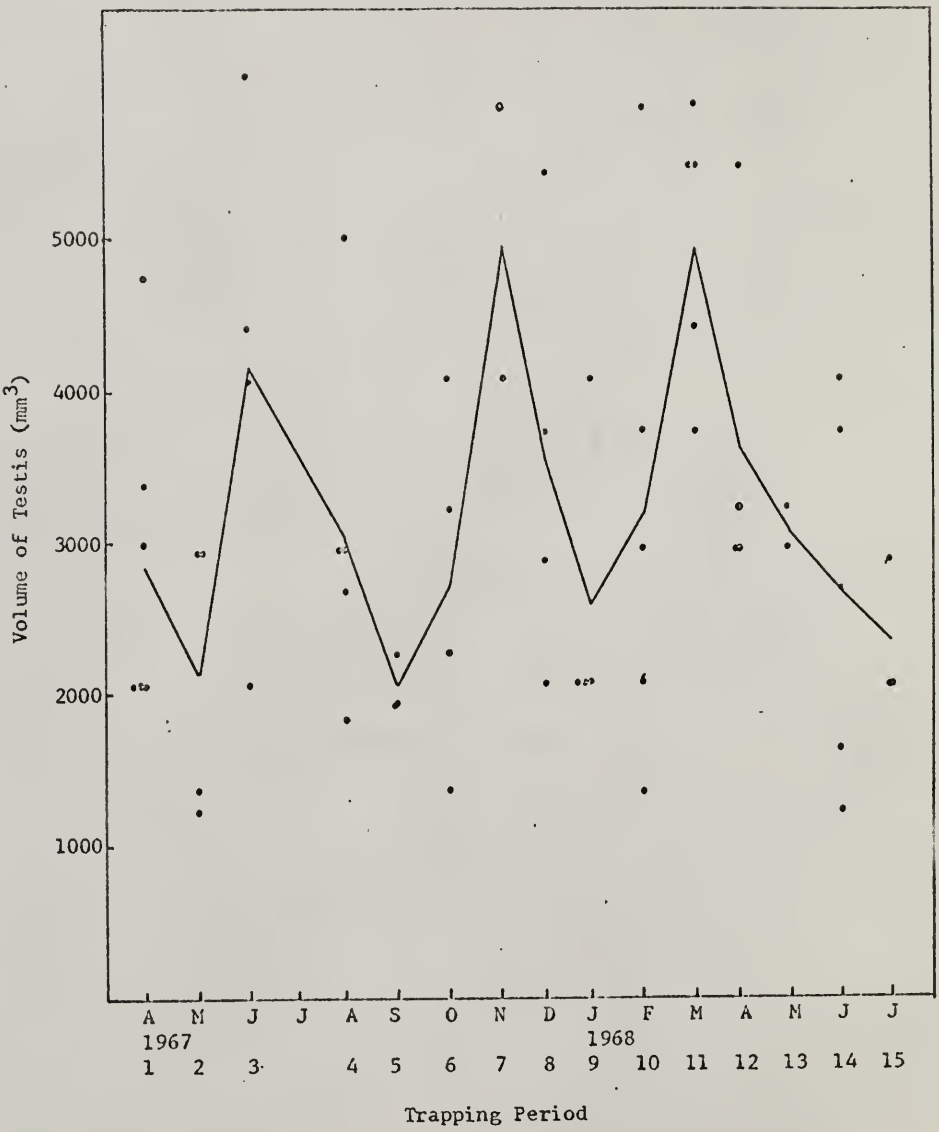

Fig. 2. Testicular volumes throughout the trapping periods. Solid line connects mean values.

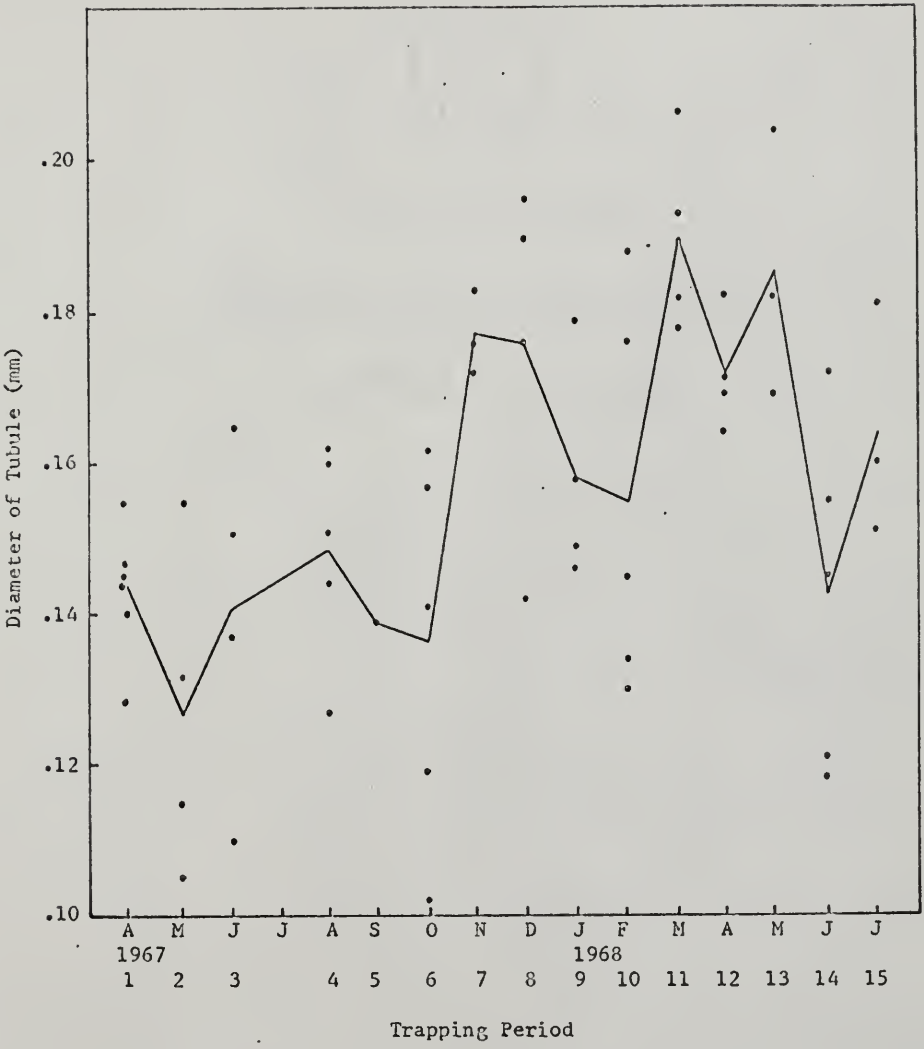

Fig. 3. Diameters of the testis tubules throughout the trapping periods. Solid line connects mean values.

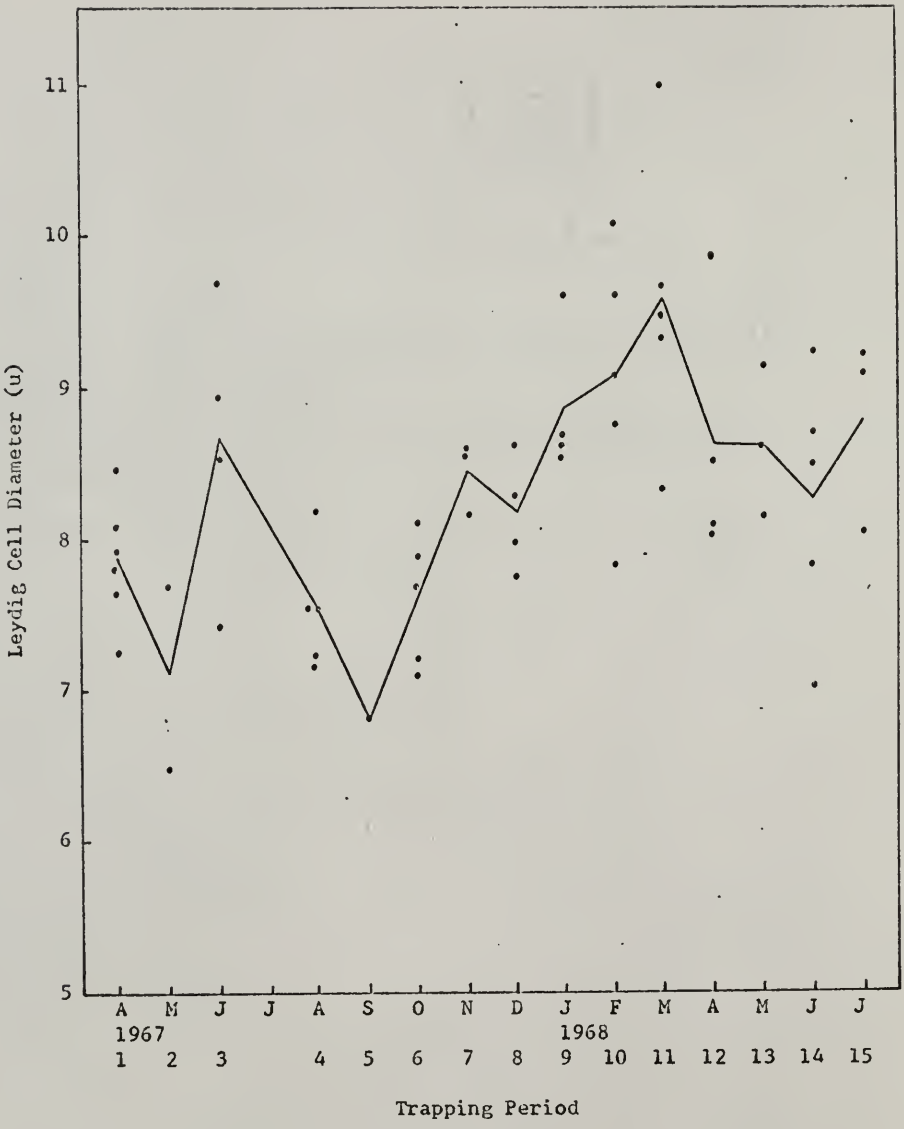

Fig. 4. Diameters of the Leydig cells throughout the trapping periods. Solid line connects mean values.

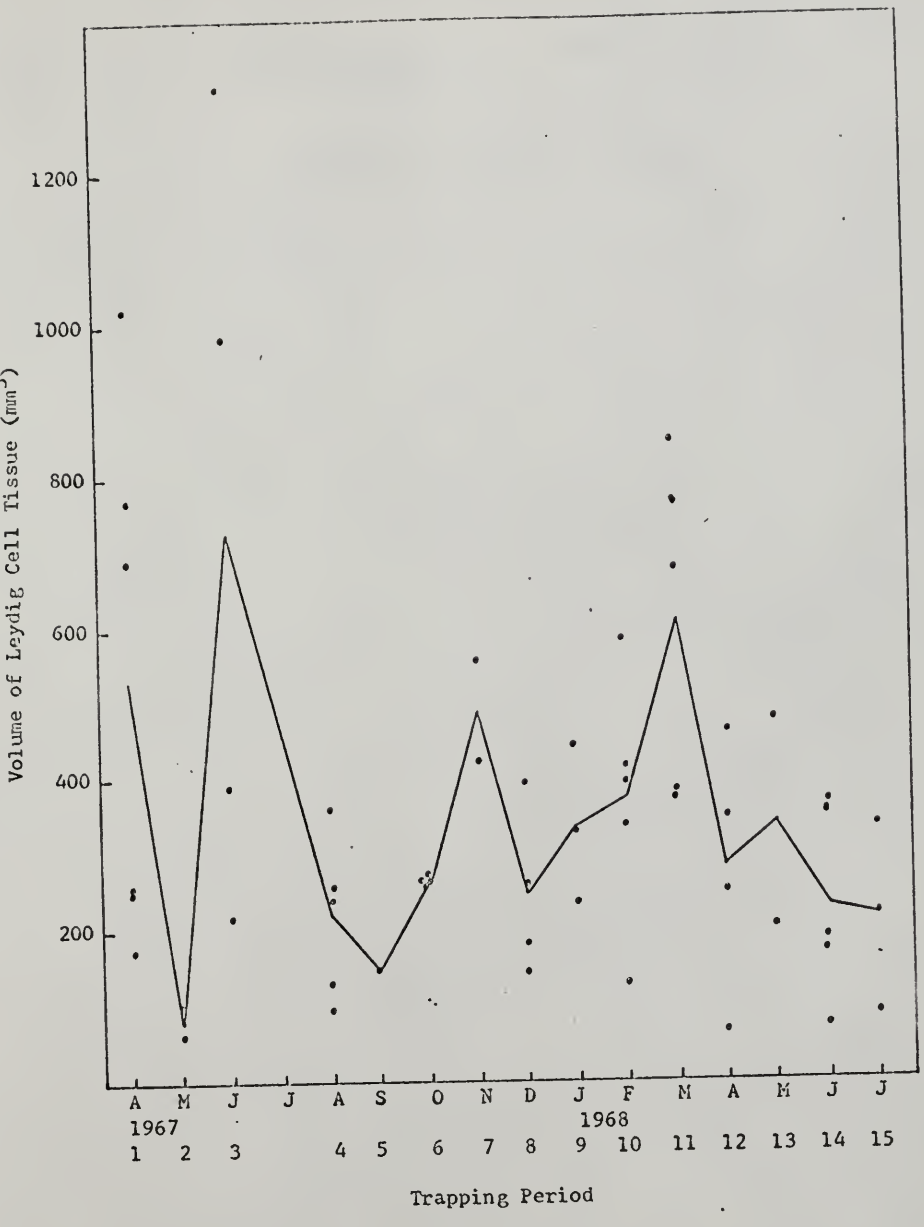

Fig. 5. Volumes of Leydig cell tissue throughout the trapping periods. Solid line connects mean values.

Although the F ratio for spermatogenesis was not significant, the cycle outlined by the means in Fig. 6 seemed to correspond to those of the previous parameters. The lack of significance here was caused by the great amount of variation around each of the means.

Changes in the Epididymis

Changes in the ratio of epididymal cell height to tubule diameter were, as in the Leydig cells, mirror images of the changes in tubule diameter. Fig. 7 therefore shows significant peaks in epididymal tubule diameter at Period 3 and Periods 11 and 12. Corresponding significant lows occurred in the ratio of cell height to tubule diameter.

Changes in the Accessory Glands

The peaks of the accessory gland measurements were less well defined than those in the epididymis and testis parameters. A low early peak was generally succeeded by a long, relatively gentle curve. In the dorsolateral prostate (Fig. 8), the first peak was reached at Period 4, whereas the second persisted from Period 9 to Period 12. The seminal vesicle cell height (Fig. 9) reached a peak in Period 3 and again from Periods 9 through 13. The seminal vesicle diameter (Fig. 10), however, did not reach a significant peak until late in the trapping period, when it remained large from Periods 11 through 13. Periods 14 and 15 might have been included in this peak, but were significantly lower than Period 13.

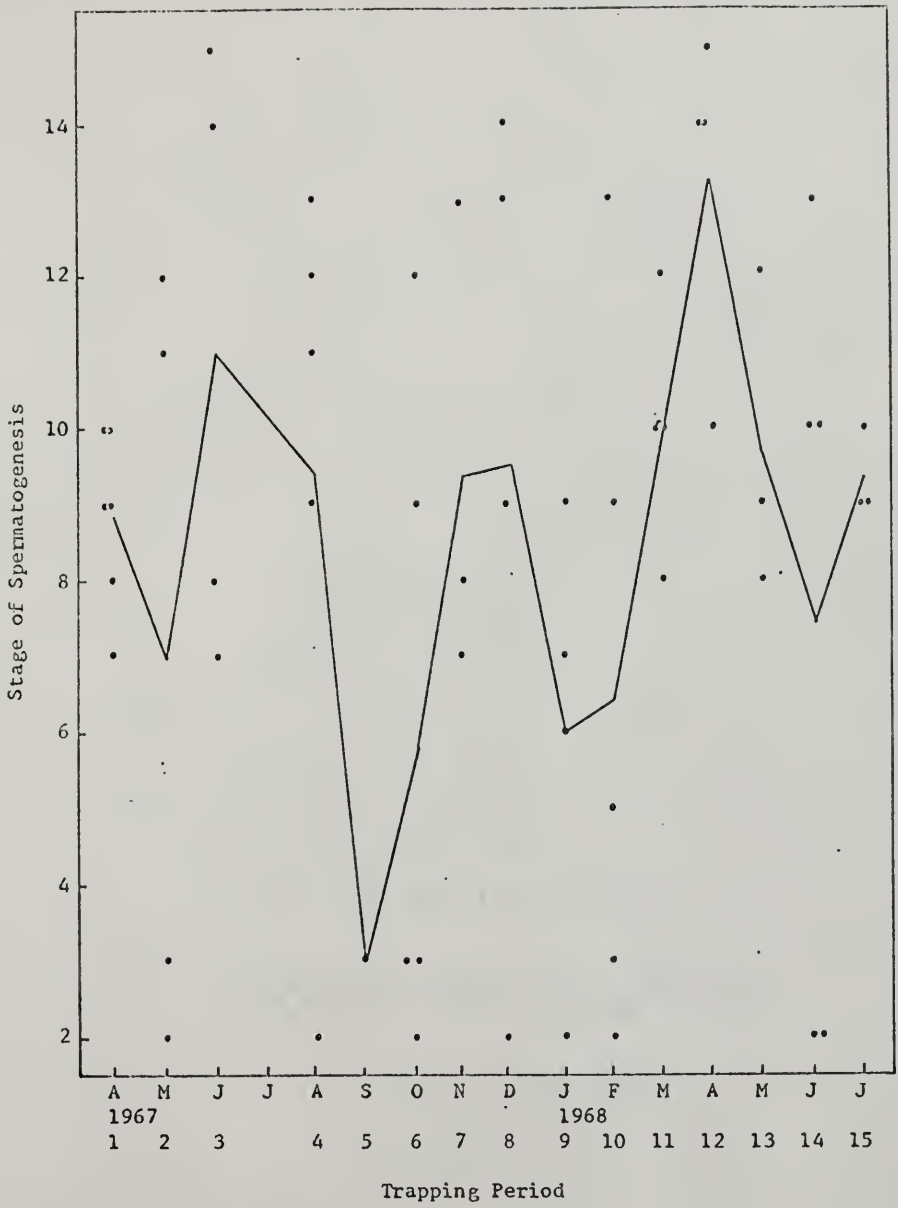

Fig. 6. Values for the stages of spermatogenesis throughout the trapping periods. Solid line connects mean values.

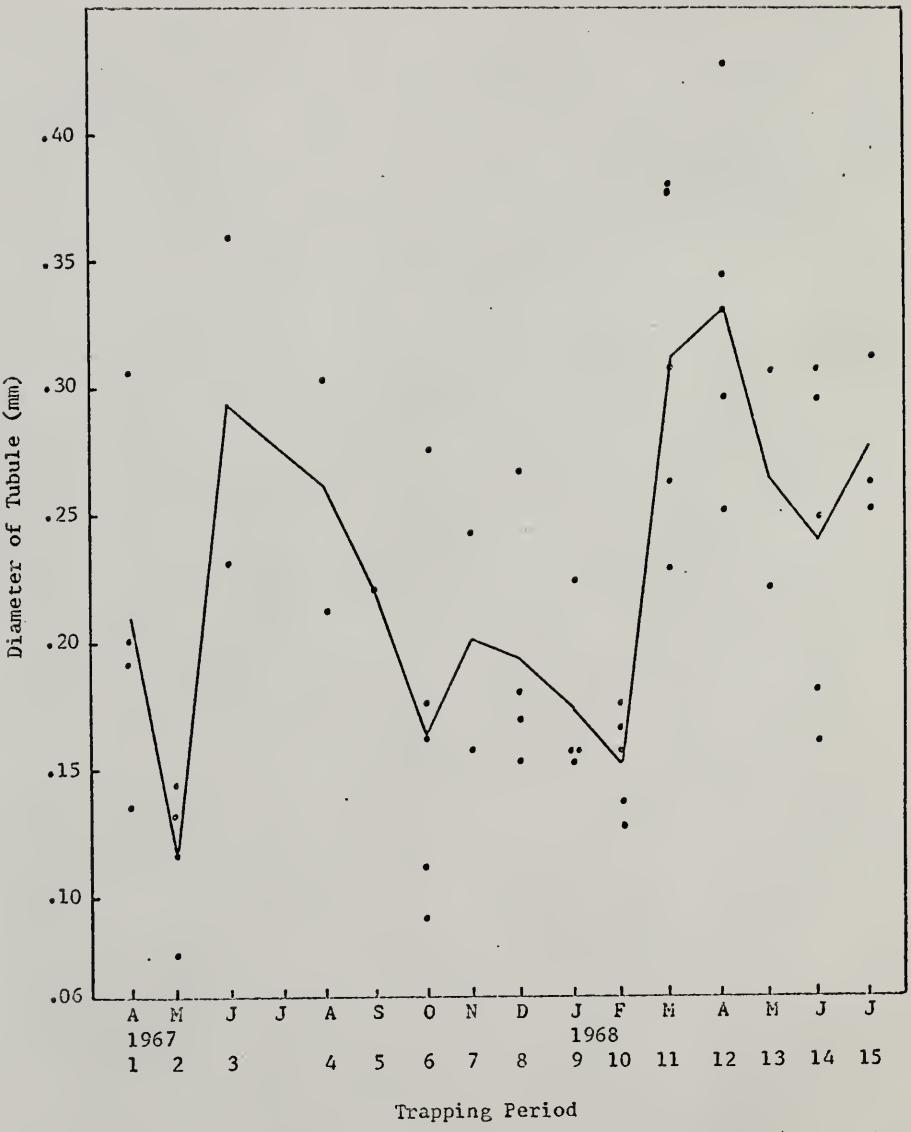

Fig. 7. Diameters of the epididymal tubule throughout the trapping periods. Solid line connects mean values.

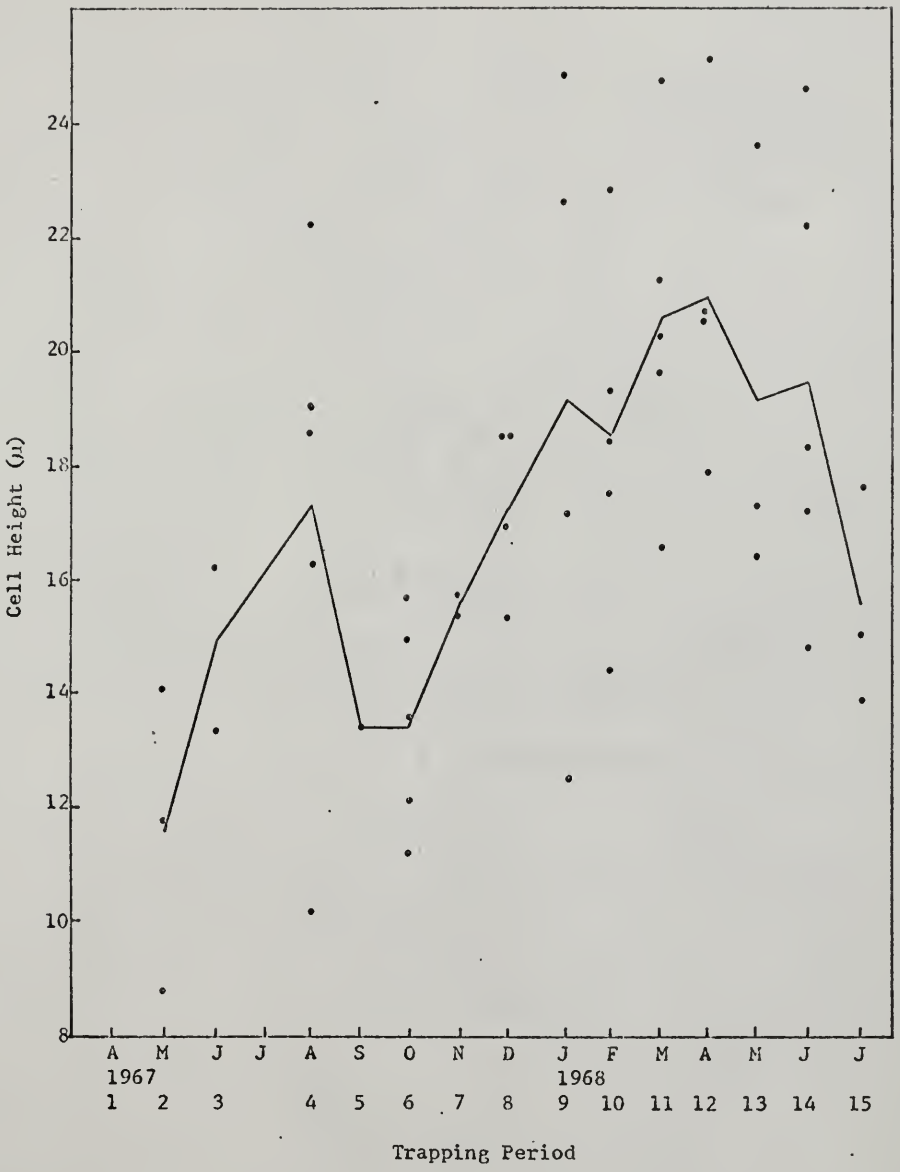

Fig. 8. Cell heights in the dorsolateral prostate gland throughout the trapping periods. Solid line connects mean values.

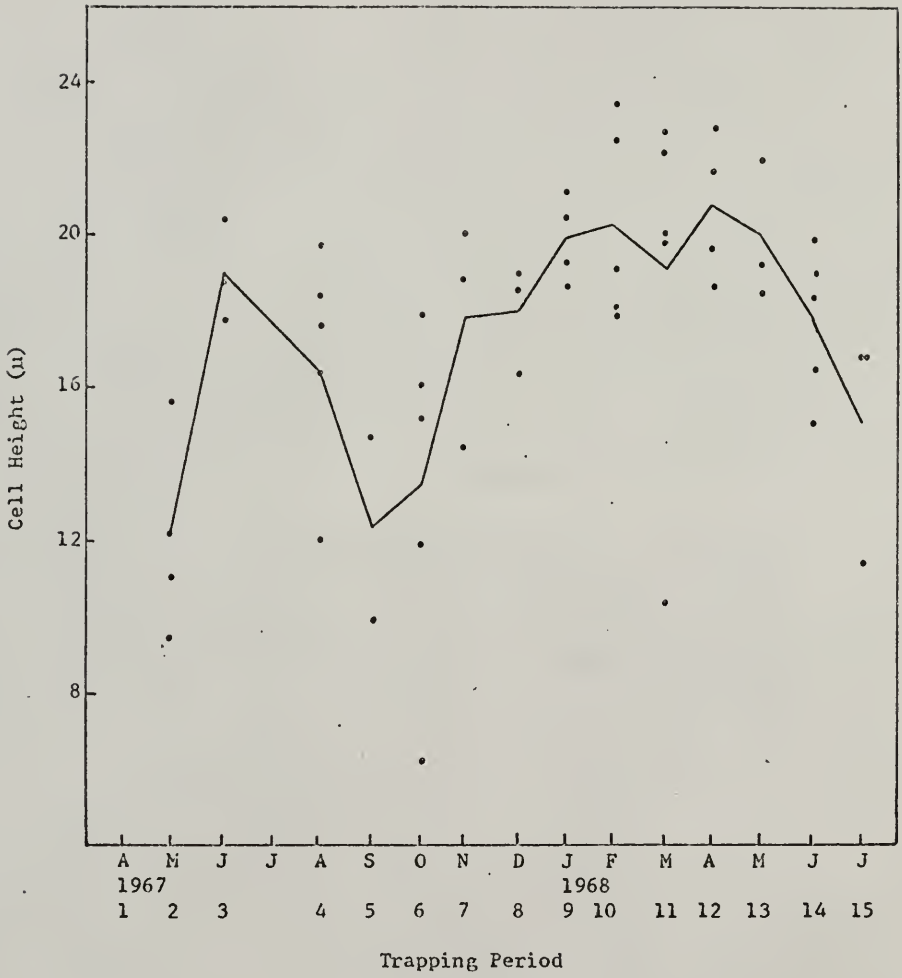

Fig. 9. Cell heights in the seminal vesicles throughout the trapping periods. Solid line connects mean values.

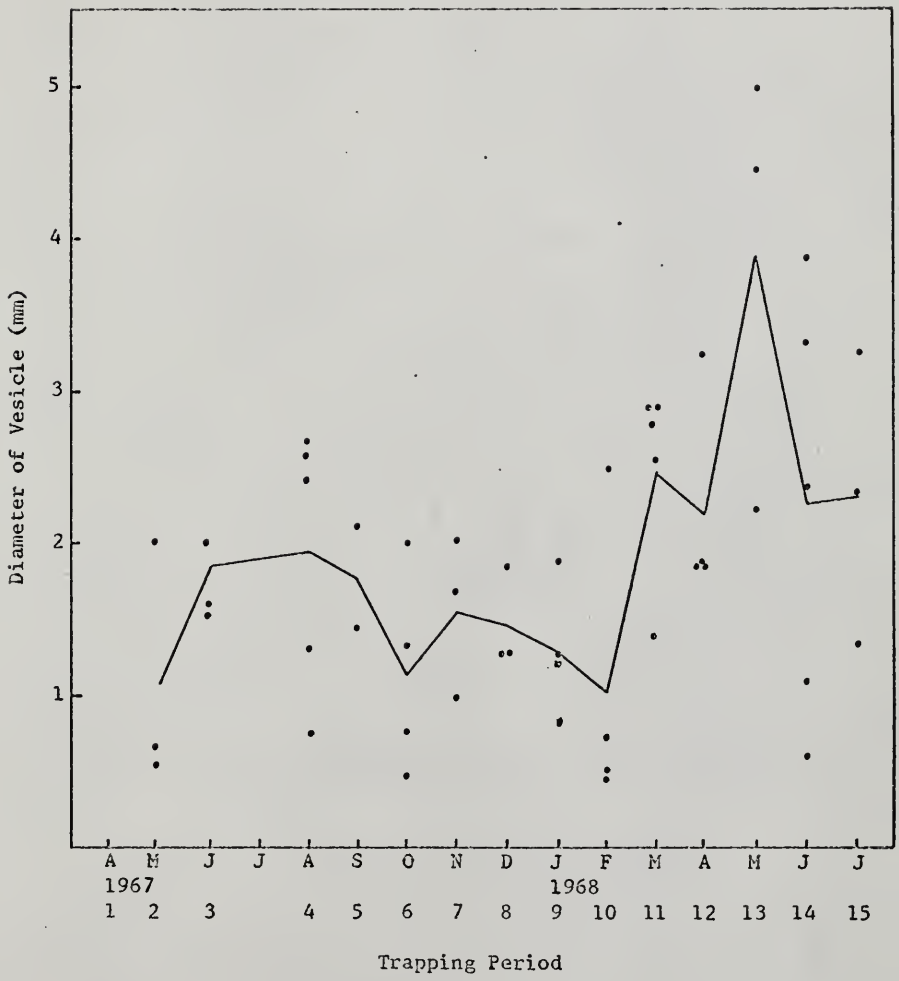

Fig. 10. Diameters of the seminal vesicles throughout the trapping periods. Solid line connects mean values.

Comparison of Changes in Reproductive Organs with Environmental Features

Table 7 demonstrates considerable overlapping of the peaks among the parameters. The interpretation of this synchrony requires a close look at the environmental features.

Fig. 11 is a summary of the monthly averages of soil temperature maxima and minima and of rainfall. Fig. 12 shows the average monthly values of soil moisture, a function of both rainfall and temperature. Periods of high soil moisture occurred at times of high rainfall and high temperatures, and at times of moderate rainfall and low temperatures. Figures 13 and 14 were abstracted from the previous two figures and present the mean of the environmental histories of the pocket gophers within each period. Only the two-week-prior soil temperature history and the four-week-prior soil moisture history are shown. The two main reproductive peaks matched those of soil moisture fairly well, and corresponded to intermediate periods of increasing soil temperature.

In spite of this visual correspondence, soil moisture showed no correlation with any one of the reproductive parameters, and soil temperature was correlated at the .05 level of significance with only five of the 14 parameters. Since the initial analyses tested only relationships between single factors -- a highly artificial situation -- a multivariate analysis was chosen that would account for interaction among the two sets of parameters.

The results of the canonical analysis are shown in Table 8. Of the four programs that were run, only the results from the one showing the highest correlation -- the combination of soil moisture two weeks prior to capture and soil temperature four weeks prior to capture --

Table 7. Distribution of significant peaks among the parameters

Parameter	Trapping Period 1967 1	2	3	4	5	6	7	8	1968 9	10	11	12	13	14	15
Testis Volume			X				X				X				
Testis Tubule Diameter							X	X			X				
Leydig Cell Diameter			X								X				
Nucleus-Cell Ratio of Leydig Cell			X								X	X	X		
Leydig Cell Tissue Volume	X		X				X				X				
Epididymal Tubule Diameter			X								X	X			
Epididymal Cell-Tubule Ratio			X								X	X			
Dorsolateral Prostate Cell Height			X						X	X	X	X			
Seminal Vesicle Cell Height			X								X	X	X		
Seminal Vesicle Diameter											X	X	X		

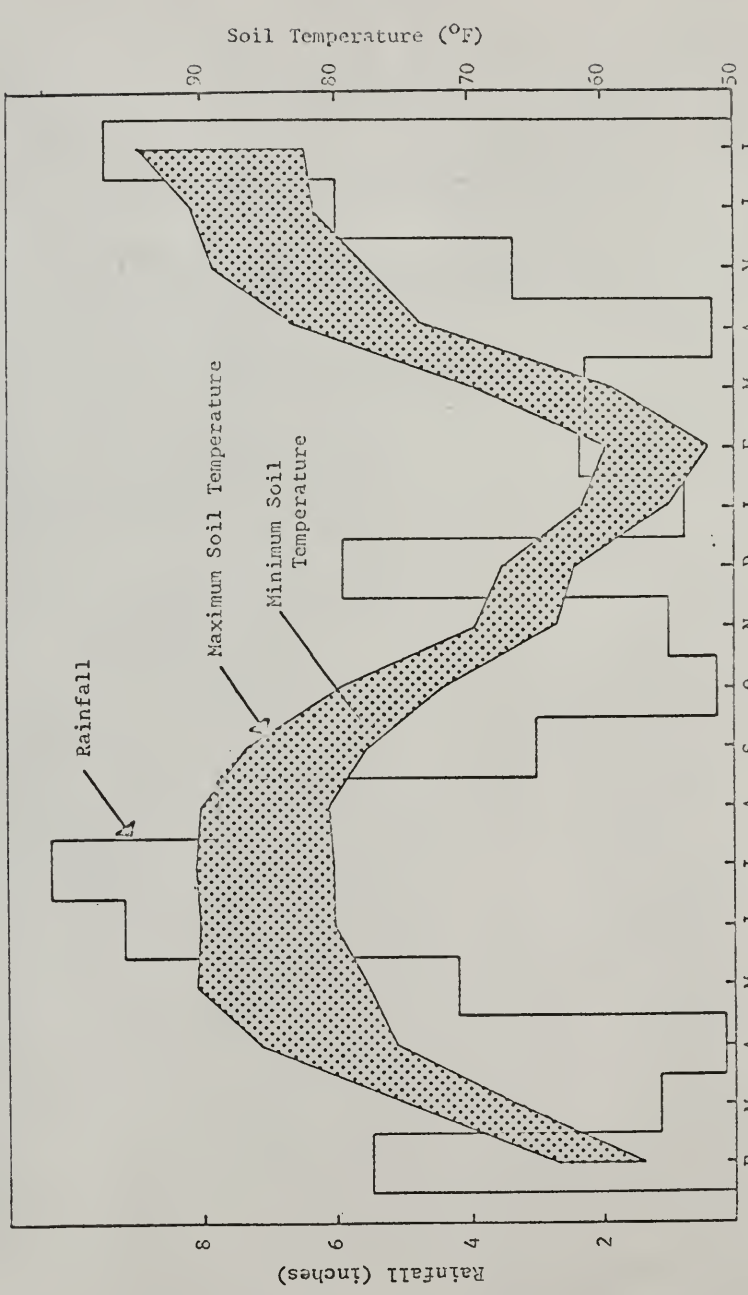

Fig. 11. Monthly rainfall and average maximum and minimum soil temperatures. (Data obtained from Agronomy Farm Weather Station, Gainesville, Florida)

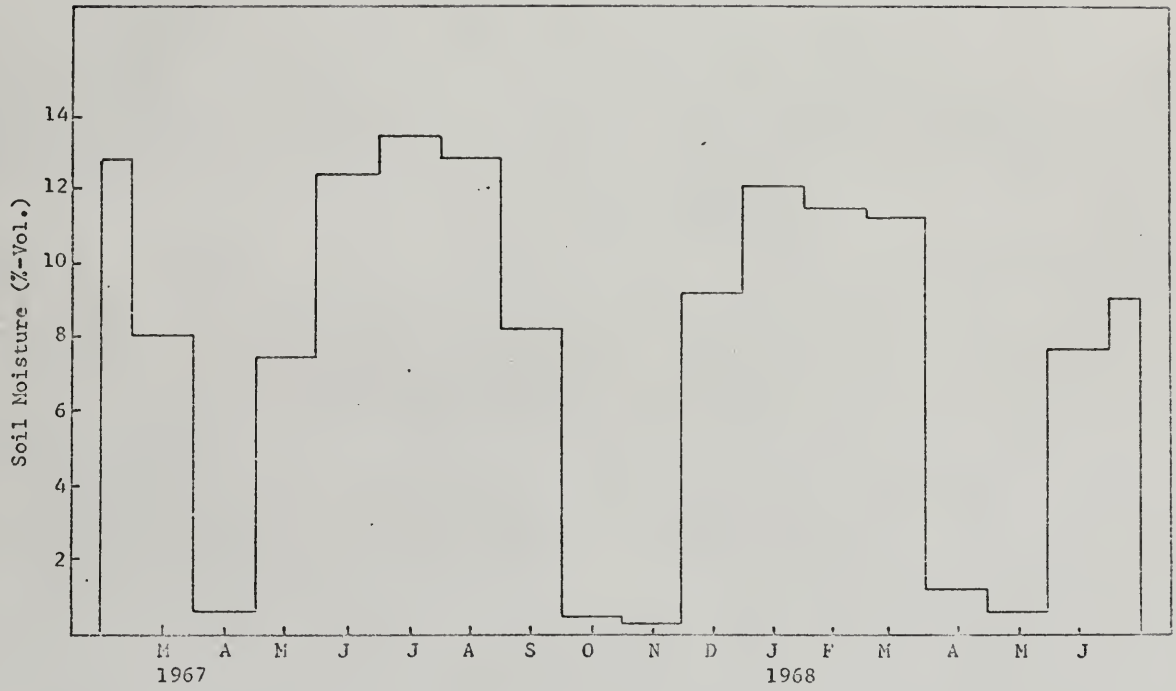

Fig. 12. Average monthly soil moisture

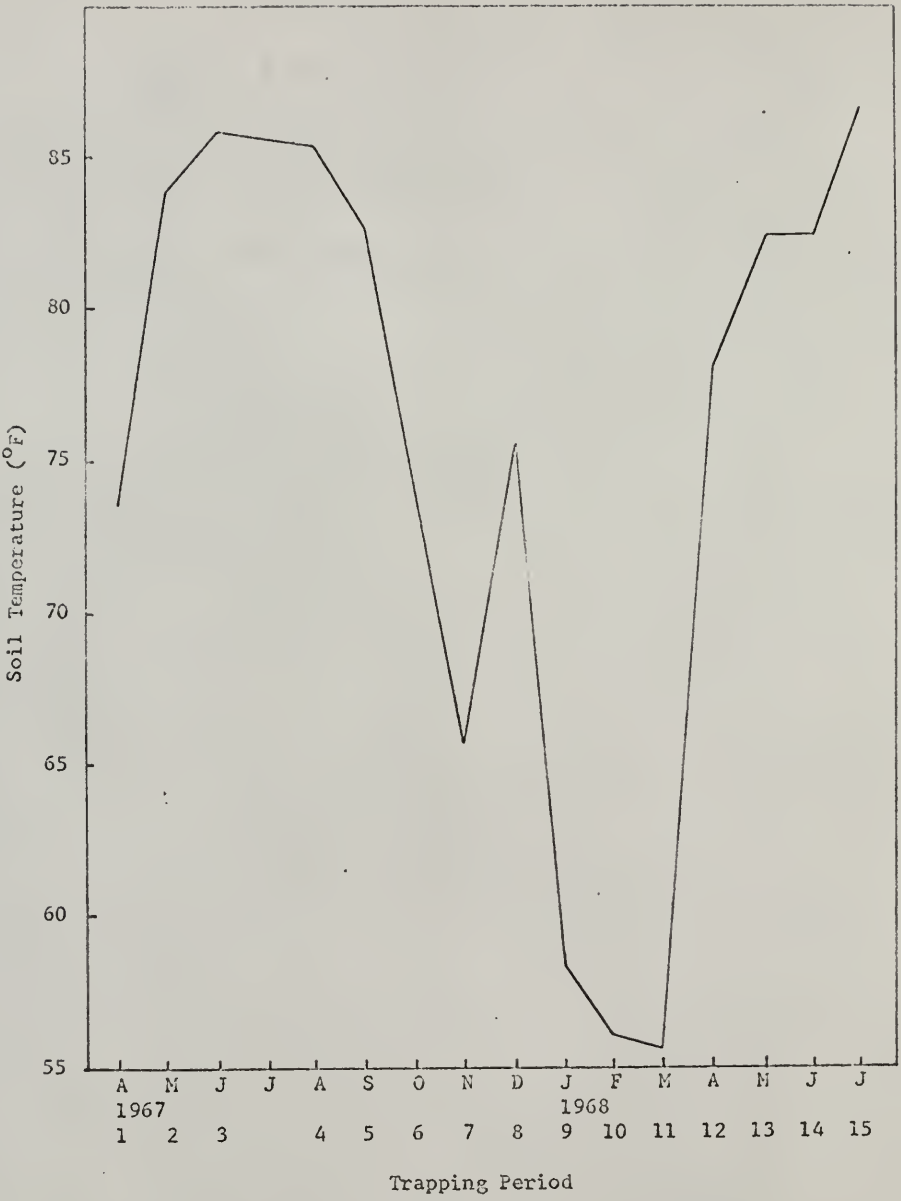

Fig. 13. Average soil temperature histories of pocket gophers within each trapping period for two weeks prior to their capture

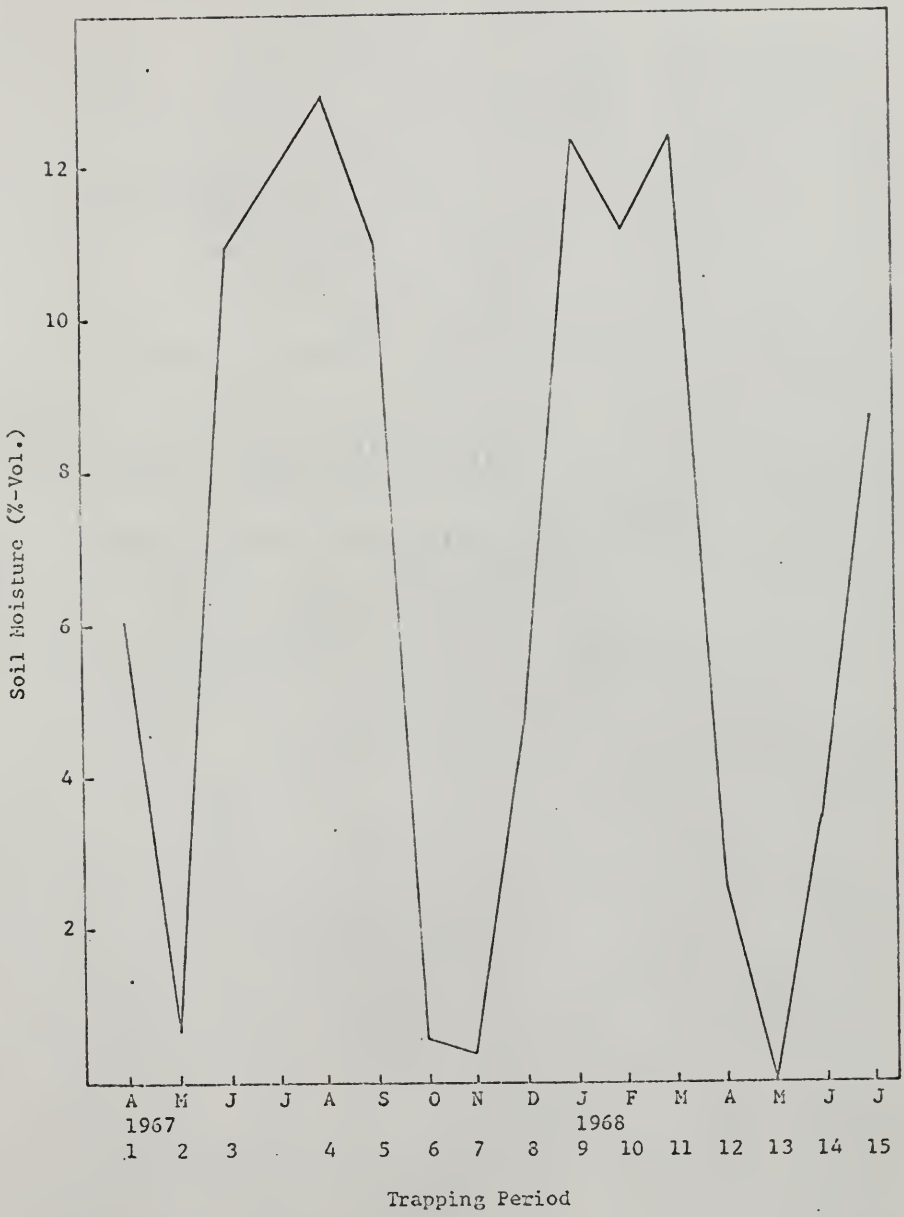

Fig. 14. Average soil moisture histories of pocket gophers within each trapping period for four weeks prior to their capture

Table 8. Results of canonical analysis

	Canonical Correlations 1 .8512	2 .4306
Parameter	Loadings	
Soil Moisture -- 2 weeks	0.0954	1.0802
Soil Temperature -- 4 weeks	1.0330	0.3299
	***	
Testis Volume	-0.4592*	0.1088
Testis Tubule Diameter	-0.5698*	-0.4652*
Leydig Cell Diameter	-0.3206*	0.5099*
Nucleus-Cell Ratio of Leydig Cell	0.0386	-0.3792*
Leydig Cell Tissue Volume	-0.1977	-0.1687
Epididymal Tubule Diameter	0.0806	0.1770
Epididymal Cell-Tubule Ratio	-0.5717*	0.8601*
Seminal Vesicle Diameter	0.3826*	0.6500*
Seminal Vesicle Cell Height	-0.3665*	-0.9869*
Dorsolateral Prostate Cell Height	0.2438	0.2326

*indicates a highly important loading

are listed. The next highest correlation was from the combination of soil moisture four weeks prior and soil temperature four weeks prior. The canonical correlation coefficients in the second set were quite close to those in the first, suggesting that there is little difference in the effects of soil moisture between the two- and four-week values. The other two programs had coefficients significantly lower than these.

The canonical correlation showed that soil temperature over a four week period was much more important than soil moisture in relating the two sets of variables. The second correlation implied that some of the variables were definitely affected by soil moisture, but the great difference between the two coefficients indicated that these second values were not so reliable. No formal significance test was performed; however, all coefficients greater than 0.3000 were considered highly important.

Not all the reproductive organs seemed to respond with the same magnitude of change, or even in the same direction. Three of the parameters did not show significance in either correlation: dorsolateral prostate cell height, epididymal tubule diameter, and Leydig cell tissue volume. For the most part, however, the relationship was an inverse one: the measurements of reproductive organs decreased with an increase in soil temperature. Most of the second set of coefficients were reversed, indicating that the measurements increased with an increase in soil moisture. Both the testis tubule and seminal vesicle cell height decreased with the increase in soil moisture, however. Because a decrease in the ratios of Leydig cell and epididymis measurements represented an increase in activity, their signs should be interpreted in the reverse direction.

Immature Animals and Pregnant Females

Little is known about the gestation and weaning periods of G. pinetis. Howard and Childs (1959) estimated the gestation period of T. bottae to be 30 days, but Schramm (1961) observed two pocket gophers (T. bottae) giving birth only 19 days after copulation; their young weighed 2 - 3 g. Barrington (1940) found that the weight of three newborn G. pinetis averaged 5.1 g, and he estimated that the gestation period in this species is 30 days. Miller (1946) reported that weaning takes place in T. bottae after 35 to 37 days, and sexual maturity is reached after three months. Wing (1960), however, indicated that from the evidence provided by seasonal percentages, G. pinetis of both sexes take nearly six months to reach sexual maturity. Wing (1960) described the largest embryo in her collection as measuring 39 mm from crown to rump. After estimating the gestation period to be one month and assuming that the maximum crown-rump length of an embryo is 40 mm, an approximation of each embryo's age in days may be calculated. This information can then be used to estimate the date of conception.

Criteria for separating immature and mature individuals in this study were, for females, the resorption of the pubic symphysis (Hisaw, 1923), and, for the males, the size of the testis. Immature males on this basis ranged in weight from 65 - 163 g, and immature females, from 60 - 146 g. If two months is arbitrarily chosen as the youngest age at which a pocket gopher can be trapped, a hypothetical age structure may be set up as follows:

Males		Females	
Weight g	Age months	Weight g	Age months
65	2	60	2
90	3	80	3
115	4	100	4
140	5	120	5
165	6	140	6

Again estimating the gestation period to be about one month, the date at which conception took place can be calculated by adding one month to the age and subtracting the total from the month of capture.

Fig. 15 shows the distribution of approximate conception dates for both pregnant females and immature animals over the total trapping period. Table 9 compares the average soil moisture and soil temperature histories, averaged over two-week and four-week periods, respectively, before each projected conception date in both the immature and the pregnant female groups.

Quarterly Sample

A total of sixty pocket gophers was trapped during the quarterly sampling periods, 15 from each period. Fig. 16 shows the pattern of monthly rainfall and soil temperature during this period.

Table 10 gives the percentage of animals in each sex group, age group, and general reproductive stage. The reproductive condition of the females was based on the size and vascularity of the uterus; males were considered to be in active reproductive condition if the testes

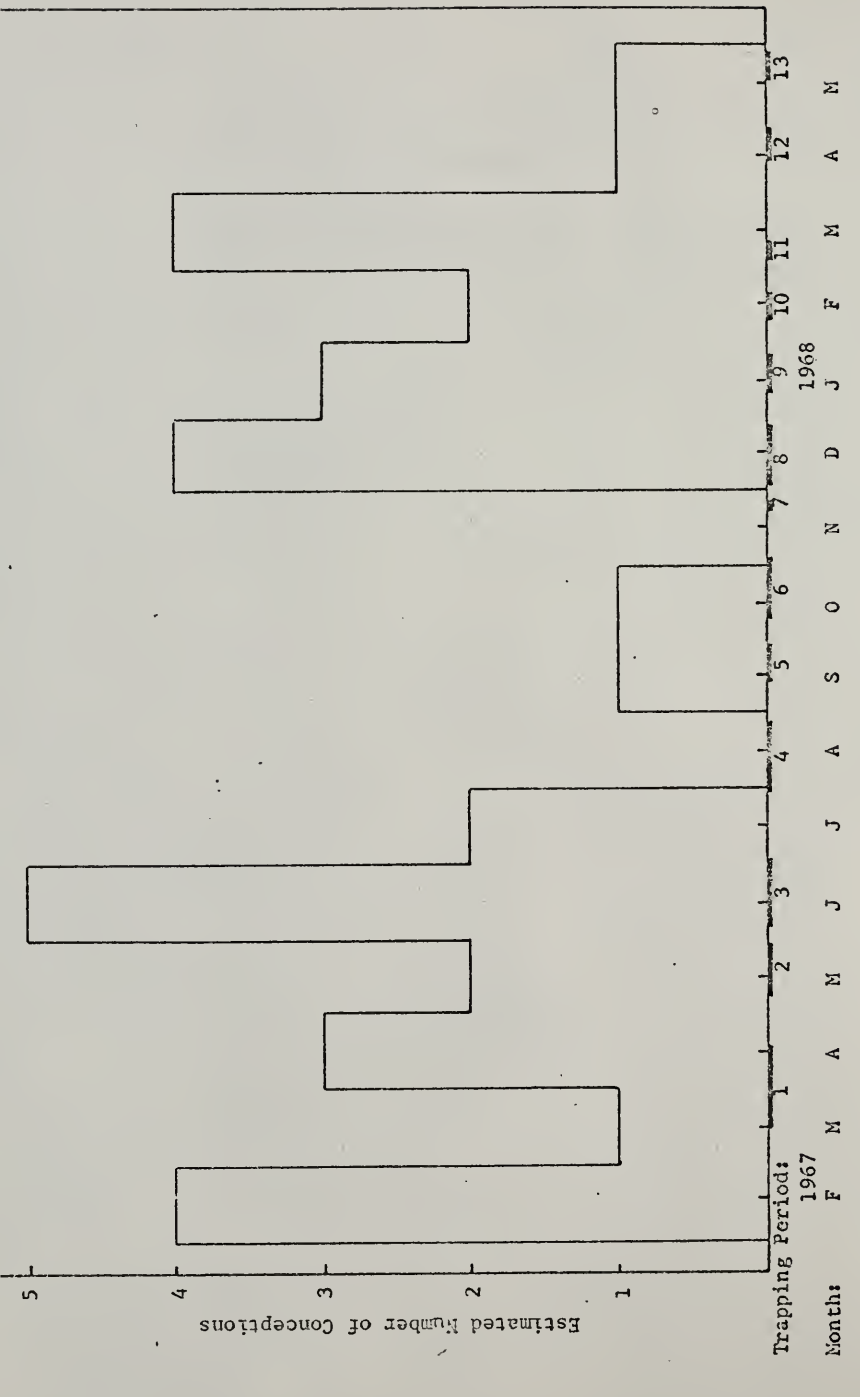

Fig. 15. Periods of reproductive activity based on all projected conception dates

Table 9. Mean values of environmental parameters over the specified periods of time before approximate conception dates. Overall means are calculated from capture dates of males throughout the year.

Group	Soil Moisture (Two-Week Average) %-Vol.	Soil Temperature (Four-Week Average) ^{o}F
Immature Animals	9.62	69.78
Pregnant Females	7.24	71.73
All Estimated Conception Dates	8.67	70.52
Overall Means	$6.82 \pm .68$	$74.24 \pm .14$

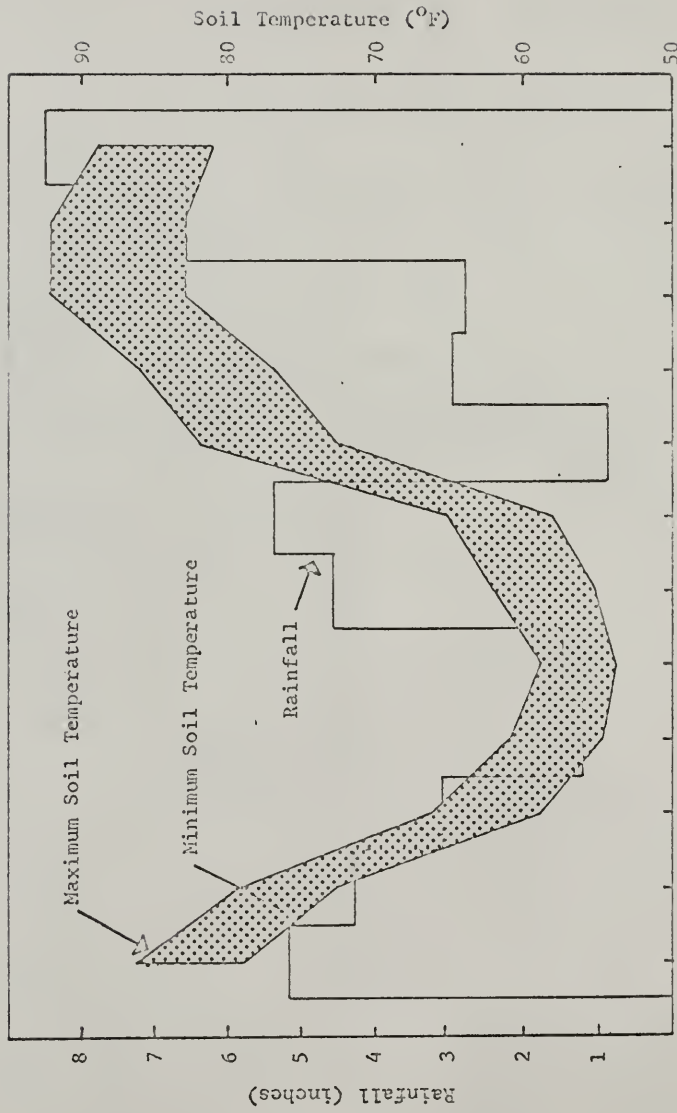

Fig. 16. Monthly soil temperatures and rainfall during quarterly sampling period

Table 10. Sex, age, and reproductive status of animals captured in each quarterly sample

Group	Quarterly Sample[1] 1	2	3	4
Percent Immature Animals	7	13	20	60
Percent of Adult Females which are Pregnant	0	28	50	0
Percent of Adult Males and Females in Active Reproductive Condition	36	85	73	33

[1]1: 11/9/68 - 11/12/68

2: 2/20/69 - 2/23/69

3: 5/26/69 - 6/5/69

4: 8/11/69 - 8/13/69

were enlarged and dark, and if the epididymal tubules were easily visible to the naked eye.

The projected conception dates for both immature animals and pregnant females were combined and are shown in Fig. 17.

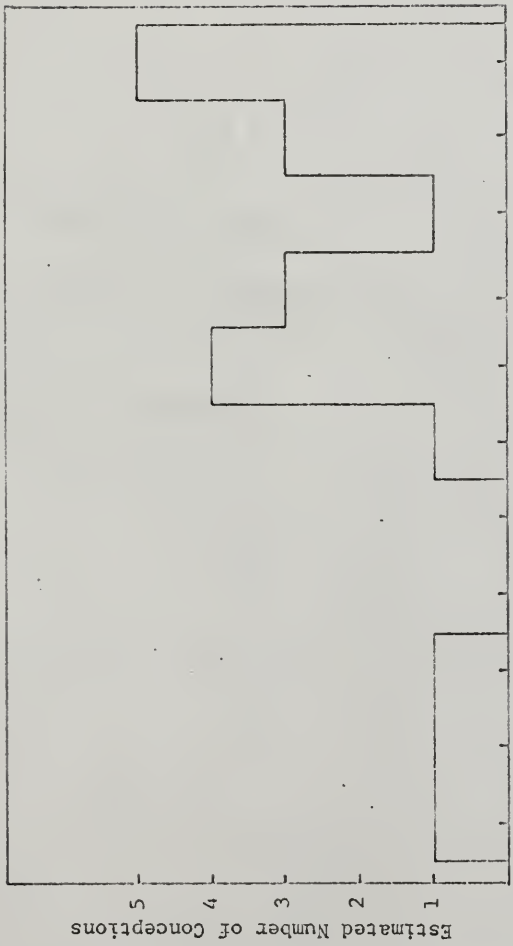

Fig. 17. Periods of reproductive activity according to projected conception dates in quarterly samples

DISCUSSION AND CONCLUSIONS

The reproductive organs of the male pocket gopher show seasonal changes that are related to changes in soil temperature and soil moisture. The possibilities that males maintain active spermatogenesis all year or that cessation or fluctuations in spermatogenesis occur synchronously throughout the population were, therefore, eliminated. Instead, it appears most likely that while some periods seem optimum for all male pocket gophers, unfavorable periods do not cause abridgement of reproductive activity to the same degree in all the males in the population. In reaching this conclusion, much insight was gained into age-dependent changes. Particularly valuable was information provided on changes within the testis.

Even after sexual maturity is reached, the testis volume, for example, was found to increase with age. This would be due to two factors: increases in testis tubule size and in interstitial tissue. Testis tubule diameter also increased with age, but the increase in volume of the total Leydig cell tissue proved not to be significant. The percentage of Leydig cell tissue volume to total volume seemed to decrease, and the diameter of the Leydig cell itself decreased with age. The diameter of the Leydig cell nucleus also decreased significantly with age relative to the diameter of the entire cell.

This situation suggests that the rate of Leydig cell division accelerates at puberty, accompanied by a disproportionate increase in the growth rate of the cytoplasm relative to the nucleus. Although

the post-pubertal Leydig cell is larger than the pre-pubertal cell, its diameter decreases with age. Conaway (1959) recorded a similar phenomenon in the eastern mole (Scalopus aquaticus). The infantile testis was characterized by abundant but small Leydig cells with very little cytoplasm. Early increases in testis volume were due primarily to increases in Leydig cell volume until testis tubule enlargement and the concurrent beginning of spermatogenesis became significant at puberty. After this stage, the diameter of the Leydig cell decreased by one-third, increasing again slightly at the end of the active breeding period. The cell diameter was found to be largest during the period of sexual inactivity.

In studies on the rat, Clegg (1966) found that the number of Leydig cells actually declined following puberty. He suggested that the accessory reproductive organs, which showed their maximum growth acceleration at that stage, were more sensitive to the androgens being produced by the Leydig cells.

Albert (1961) indicated that Leydig cells in bulls also decreased in size from 5 to 15 years, after an initial increase in both number and size from two years to five years. According to the same source, the number of Leydig cells in man declines with age.

The percentage of Leydig cell tissue volume in the hippopotamus testis decreased from 64% in the very young animal to 32% at puberty, remaining steady through old age (Laws and Clough, 1966).

The decrease in size of Leydig cells in pocket gophers as well as the decrease in percentage of total testis volume is, therefore, not an unusual situation. Factor 3 related the increase in Leydig cell diameter with the rise in activity of the accessory glands, as

well as with the increase in the diameter of the epididymal tubules relative to the epididymal cell heights. Unlike the situation described earlier in moles (Conaway, 1959), hormone secretion is presumably associated with both a temporary increase in diameter of the Leydig cells as well as an increase in the ratio of cytoplasm to nucleus. This evidence, together with the associations in Factor 2 which were mentioned earlier, leaves little doubt that a cyclic increase and decrease of both Leydig cell diameter and of the tissue volume is imposed on the long-term pattern of diminution.

The height of the epididymal cell was found to increase in relation to the tubule diameter with age. The cell height was also associated with Leydig cell diameter in Factor 2. The latter response corresponds to the observations summarized by Mann (1964), which showed that survival of sperm in an epididymis severed from the testis was dependent on the continued presence of the testis in the body. He concluded that the condition of the epithelium in the epididymal tubule was strongly influenced by the male sex hormone. The function of the epididymis, Mann surmised, was therefore not only to serve as a repository for sperm but also to produce a secretion somehow effective in preserving or perhaps maturing the sperm.

Well-defined cycles during the study period were found only in the testis volume, Leydig cell diameter and tissue volume, and epididymal tubule diameter and cell height. The stage of spermatogenesis was highly dependent on age, the variation in stages among the pocket gophers in each trapping period being sufficient to reject the possibility of there being a synchronized population cycle in the production of spermatozoa. Although definite cycles were detected in

the activities of the seminal vesicles and the dorsolateral prostate, they were less distinct than the cycles mentioned earlier. Moreover, activity in the prostate glands did not necessarily cease when sperm were not being produced. The seminal vesicles, however, showed a pronounced increase in activity in animals with active sperm production. Price and Williams-Ashman (1961) found that testicular hormones provided most of the control over activity of the accessory glands. They also concluded that the chief function of these glands is to secrete the seminal plasma.

Internal control of the reproductive system in the male pocket gopher seems to rest at the hormonal level. Maximum size of the Leydig cells reached two significant peaks which corresponded to similar peaks in size of the epididymis and accessory glands. The lack of complete correspondence is likely attributable to a low threshold in glandular response to testosterone. Van Tienhoven (1968) has also suggested that maintenance of secondary sex characteristics outside the breeding season may be the result of androgen secretion by extra-testicular sources such as the adrenal cortex.

Strongly influencing hormonal control, however, is environmental control. In the canonical analysis, most of the measurements taken of the reproductive organs decreased in magnitude, or otherwise denoted less activity, under conditions of high soil temperature and low soil moisture. This analysis, together with the tentative dates of conception of immature pocket gophers and embryos of pregnant females, as well as the correspondence between environmental histories and the peaks of reproductive activity, suggests that reproductive activity is greatest in periods of moderate temperature and high soil moisture.

Soil moisture is logically the less important of the two because it seldom dips below 50% of the field capacity, and therefore shows comparatively little variation. The distribution of G. pinetis may well be limited by soil moisture, since this species is found only in the well-drained sandy soils in the Southeast (McNab, 1966). The possibility mentioned earlier that excessive soil moisture might prompt a pocket gopher to dig to the surface, and perhaps more extensively underground as well (Miller, 1948), may be valid, but apparently this response does not serve as the sole cue for timing the reproductive cycle.

Temperature does affect the cycle strongly, most likely in an indirect fashion. Because the largest proportion of the burrow lies near the surface of the ground, it may be inferred that this is where the greatest amount of digging takes place. The pocket gopher, however, is a poor thermoregulator and therefore cannot long withstand the heat stress of digging in a shallow, warm burrow. Gunther (1956) proposed a behavioral mechanism in which the gopher makes use of deeper, cooler parts of its burrow system in the summer, plugging some of the shallow feeding tunnels. The diminished amount of digging resulting from this cessation of activity would presumably lead to diminished intraspecific contact. This regulation of activity would effectively restrict most reproductive activity to certain times of the year.

The reproductive cycle gives no evidence of being strictly timed. Spermatogenesis, for instance, was found to be non-cyclic, at least during this study period. A more careful study of the reproductive tract itself might reveal storage of the sperm at some place along

the ductus deferens. The great variation in sperm production at any one time convincingly suggests that the reproductive cycle is not a strict endogenous rhythm, but is influenced by environmental fluctuations and could be triggered by the right circumstances -- i.e., favorable environmental conditions and the presence of a receptive female -- at any time of the year. Since there was a low level of reproductive activity that continued throughout much of the study period, at least some random encounters in the "off season" might be fruitful.

Probably little selection pressure operates to channel reproduction into a strictly timed cycle. Food is certainly most abundant in the spring months but is still available, particularly in a grassy area such as Stengel Field, in sufficient quantity throughout the year so that a newborn gopher would not starve. Contact between pocket gophers of the same sex frequently results in harmful injuries to one or both animals (Miller, 1964; Vaughan, 1962). Such contact, if it should occur between a male and female, might also be harmful if neither were in active reproductive condition. It would obviously be advantageous to a pocket gopher to be capable of mating should an individual of the opposite sex be encountered. However, expending energy toward maintaining the reproductive system in an active state during periods of diminished intraspecific contact could also be wasteful. Evidence from one population of pocket gophers indicates that the greatest number of the individuals reaches a peak in reproductive activity when maximum contact is expected, but seldom does the entire population regress to an inactive state at other times.

SUMMARY

The reproductive cycle in the male pocket gopher (Geomys pinetis) is closely correlated with soil temperature and to a lesser degree with soil moisture, but does not seem to be strictly controlled by either of these parameters. Very warm external temperatures and low soil moisture appear to restrict activity within the burrow at certain times of the year, and accordingly reduce intraspecific contacts. Maximum reproductive activity corresponds to those environmental conditions that create a friable soil.

Internal seasonal changes in the reproductive organs and glands are presumably in turn effected by hormones, particularly testosterone, which is secreted by the Leydig cells. Leydig cells in adult pocket gophers are larger in diameter and more numerous than those in immature animals. A net decrease in both size and number of Leydig cells occurs after puberty, but the cells still increase in diameter at times of increased reproductive activity, suggesting increased hormonal production. This cyclic change is correlated with increases in the height of the secretory epithelium in the accessory glands and in the epididymis.

Spermatogenesis was found to be non-cyclic during the study period. Although the mean spermatogenic values for each period followed a cyclic pattern similar to the one outlined by the Leydig cell diameter, the variation about the means was too great to allow statistical significance. Sperm production throughout the population therefore con-

tinued at least at a threshold level throughout the study period, and reproductive activity at the organ level was restricted more by the condition of the accessory glands and the epididymis than by the availability of sperm.

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APPENDIX

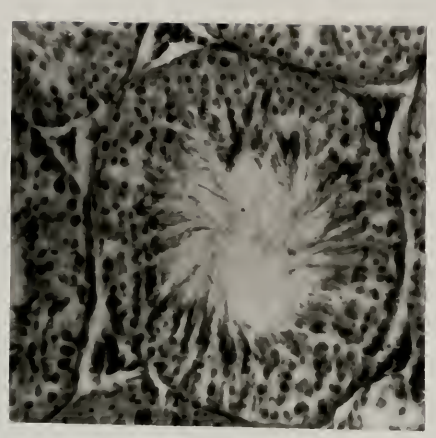
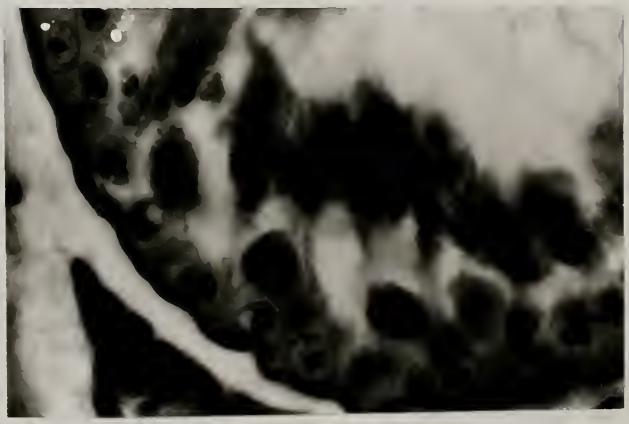


Fig. 18. Level 8 testis (100X)

Fig. 19. Level 3 testis (430X)

Fig. 20. Level 7 testis (430X)



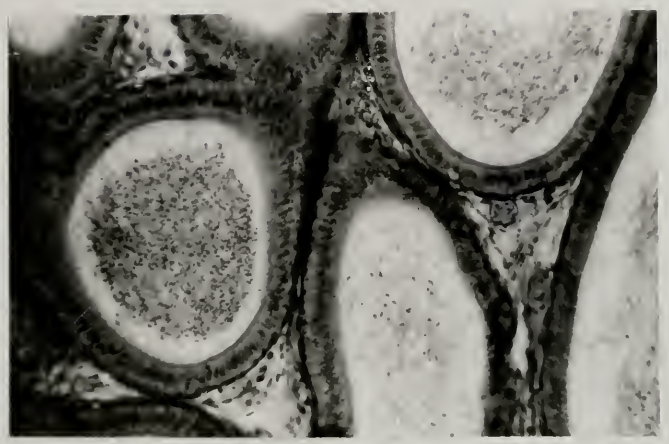


Fig. 21. Epididymal tubules from a male in active reproductive condition. The lumina are filled with sperm. (100X)

Fig. 22. Epididymal tubules from inactive male (100X)

Fig. 23. Actively secreting acinus from dorsolateral prostate gland (430X)

Fig. 24. Inactive acinus from dorsolateral prostate gland (430X)

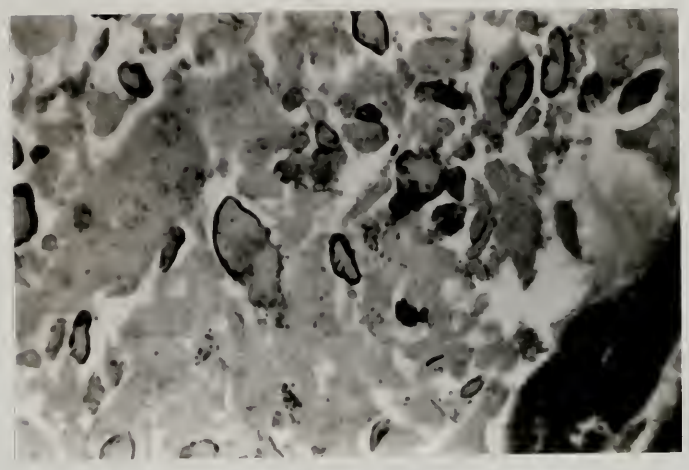


Fig. 25. Lumen and part of cell boundary from an active seminal vesicle (100X)

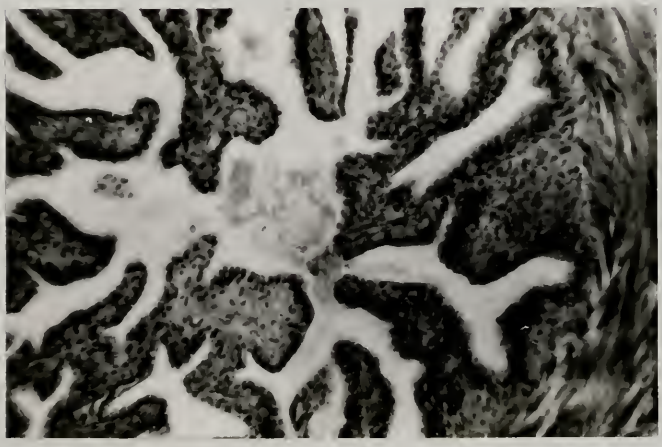


Fig. 26. Inactive seminal vesicle (100X)

BIOGRAPHICAL SKETCH

Katherine Carter Ewel was born September 30, 1944, at Glens Falls, New York. During the summer of 1961, she attended Cornell University on a National Science Foundation Summer Fellowship, and graduated from Glens Falls High School in June, 1962. In June, 1966, she received the degree of Bachelor of Arts with a major in zoology from Cornell University. During the summer of 1966, she participated in a field biology course at Tulane University on a USDHEW Environmental Training Grant and in the fall of 1966 enrolled in the Graduate School of the University of Florida. She worked as a teaching assistant in the Department of Zoology until August, 1967, and was awarded an NDEA Title IV Fellowship from September, 1967, through August, 1969. During the summer of 1968, she was enrolled in the tropical biology program at the Universidad de Costa Rica under an NSF Graduate Research Fellowship awarded by the Organization for Tropical Studies. She is currently employed as a Temporary Instructor in Zoology at Duke University while completing her work toward the degree of Doctor of Philosophy at the University of Florida.

Katherine Carter Ewel is married to John Ewel. She is a member of the American Society of Mammalogists, the Ecological Society of America, and the Society for the Study of Evolution.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1970

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